



# Effectiveness of plant growth regulators on emission reduction of greenhouse gas (Nitrous oxide): An approach for cleaner environment

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## ABSTRACT

Global climate change is one of the most prominent and complex environmental problems caused by increasing concentrations of atmospheric greenhouse gases. Rice paddies are an important anthropogenic source of nitrous oxide ( $\text{N}_2\text{O}$ ), a greenhouse gas approximately 298 times stronger than carbon dioxide. Identifying a suitable mitigation method for  $\text{N}_2\text{O}$  emissions from rice agriculture, while maintaining optimum crop productivity is strongly needed. A 2-year field study was conducted on rice to assess  $\text{N}_2\text{O}$  emissions in response to the application of different plant growth regulators (PGRs). The PGRs abscisic acid (ABA) and cytozime ( $20 \text{ mg L}^{-1}$ ), kinetin ( $10$  and  $20 \text{ mg L}^{-1}$ ) and wet tea extract ( $1:20 \text{ w/w}$ ) along with distilled water as control were sprayed at the tillering and panicle initiation stages of rice. Compared with control treatment, exogenously applied ABA and kinetin ( $10$  and  $20 \text{ mg L}^{-1}$ ) significantly ( $p < 0.01$ ) reduced  $\text{N}_2\text{O}$  emission primarily through regulation of leaf growth, stomatal frequency, and xylem vessel size of rice plants. Cytozime and kinetin improved the grain yield through efficient photosynthesis. Leaf area index, tiller number, leaf photosynthesis, and transpiration were found to be directly associated ( $p < 0.05$ ) with  $\text{N}_2\text{O}$  emission. The PGRs regulated  $\text{N}_2\text{O}$  transport by manipulating anatomical and physiological processes. Kinetin ( $10 \text{ mg L}^{-1}$ ) application can be suitable for  $\text{N}_2\text{O}$  emission reduction coupled with an increase in economic productivity. Considering the effect of  $\text{N}_2\text{O}$  on global climate change, such mitigation measures may be effective for sustaining a cleaner environment for global community.

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## 1. Introduction

Rice (*Oryza sativa* L.) is one of the most major cereal crops and the staple food for more than half of the world's population (Zschornack et al., 2016). Small farmers in developing nations cultivate 80% of the total rice cultivated globally. In India, rice cultivation covers an area of more than 40 million ha (Hardy, 2013). Rice cultivation in India is intimately associated with the country's food security. Furthermore, it is a key source of livelihood and employment. However, rice cultivation is the largest source of anthropogenic nitrous oxide ( $\text{N}_2\text{O}$ ), a potent greenhouse gas (GHG) and major contributor to ozone layer depletion (IPCC, 2014; Datta et al., 2017). Rice fields account for approximately 11% of the total global agricultural  $\text{N}_2\text{O}$  emission (Hussain et al., 2015). A

considerable amount of  $\text{N}_2\text{O}$  emission has been reported from southern and northern states of India because large areas are under rice cultivation in these states (Pathak, 2015).

The presence of excess  $\text{N}_2\text{O}$  in the atmosphere is a major global environmental concern because of the high global warming potential (GWP) of  $\text{N}_2\text{O}$ , which is 298 times higher than that of  $\text{CO}_2$  in a time horizon of 100 years. Moreover, it has a long atmospheric lifetime (UNEP, 2013). According to the report of UNEP (2013), without abatement measures,  $\text{N}_2\text{O}$  emission could almost double by 2050. The expected effects of increasing  $\text{N}_2\text{O}$  concentrations have necessitated the development of strategies to mitigate  $\text{N}_2\text{O}$  emission for achieving a cleaner environment to sustain life on earth (Houfey and Areeshi, 2015). Intensive agricultural management practices can reduce agricultural  $\text{N}_2\text{O}$  emission at the source, stabilise  $\text{N}_2\text{O}$  concentrations in the atmosphere, and mitigate climate change (Li et al., 2017).

Plants have substantial effect on  $\text{N}_2\text{O}$  production, consumption, and transport in the environments (Pihlatie et al., 2005; Wang et al., 2008). Plants play a critical role in regulating the chemical and

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physical state of the atmosphere through the exchange of biogenic GHGs. According to Del Grosso et al. (2000), the production of  $\text{N}_2\text{O}$  in soil is mainly controlled by the availability of nitrate, labile carbon compounds, and  $\text{O}_2$ , which in turns, are considerably affected by the presence of growing plants (Conrad et al., 1983).  $\text{N}_2\text{O}$  emission from agricultural plants has been widely reported; however, the transport mechanisms of  $\text{N}_2\text{O}$  through plants are not well documented (Baggs and Philippot, 2010). Recent reports have indicated that plants serve as a conduit for transporting  $\text{N}_2\text{O}$  to the atmosphere and account for up to 62% of the total emission (Zou et al., 2005; Bowatte et al., 2014; Borah and Baruah, 2016a).

Plant growth regulators (PGRs) such as abscisic acid (ABA), kinetin (KIN), and cytozime (CYT) affect several physiological properties and are reported to be crucial factors controlling the sink and source relationship in plants, thus influencing grain productivity (Ghodrat et al., 2012; Abubakar et al., 2013). Remarkable accomplishments, such as manipulating plant growth and crop yield, have been actualized using PGRs in recent years (Yan et al., 2011). Apart from commercial phytohormones, natural products, called growth stimulators, are also known for their growth-regulating effects (Andresen and Cedergreen, 2010). For example, tea, a natural product, contains phytochemicals that act as antioxidants and exhibit hormonal-like properties; these phytochemicals have been reported to stimulate various enzyme activities (Tariq and Reyaz, 2012). PGRs also play a central role in the adaptation of plants to changing environments by mediating photosynthesis, epigenetic modifications, nutrient allocation, and growth and development (Li et al., 2011). Therefore, modifying the physiological and anatomical characteristics of the plants through the application of PGRs might be an effective mitigation option for reducing  $\text{N}_2\text{O}$  emission. A 2-year field study was conducted to identify possible strategies for mitigating  $\text{N}_2\text{O}$  emission from rice cultivation. The objectives of the present study were as follows: 1) to assess the effectiveness of PGRs in reducing  $\text{N}_2\text{O}$  emission rates from rice cultivation; 2) to evaluate the effect of PGRs on the anatomical characteristics of rice plants and  $\text{N}_2\text{O}$  transport mechanisms through the plants; and 3) to determine whether the application of PGRs improves the economic/grain productivity of rice while simultaneously reducing  $\text{N}_2\text{O}$  emission rates.

## 2. Material and methods

### 2.1. Description of the PGRs

Abscisic acid is a plant growth regulator associated with seed dormancy, responses to stresses such as drought, extreme temperatures, excess light, and other growth processes (Hirayama and Shinozaki, 2007). Kinetin, the most known cytokinin, have significant role in enhancing division of cells, synthesis of chlorophyll, and apical dominance alteration in plants (Taiz and Zeiger, 2006; Aldesuquy et al., 2014). It significantly improves the plants' growth under salinity, soil waterlogging, and soil acidity (Kaya et al., 2010). Cytozime a synthetic plant growth stimulator, helps in increasing photosynthetic activity, improves vegetative growth of plants, and enhance grain productivity because of the presence of gibberellic acid, auxin, and cytokinins as its chemical constituents (Abubakar et al., 2013). Tea extract prepared from tea leaves has natural growth-stimulating properties and contain polyphenols, which are well-known for their antioxidant properties and antifungal activity (Inamdar et al., 2014).

### 2.2. Field site and experimental design

The study was conducted at the north bank plain agro-climatic zone of Assam, India inside the Tezpur University campus (2642' N,

92499' E) for two consecutive seasons in 2014 and 2015. The region is subtropical humid and is characterized by moderately hot wet summers and dry winters. The soil is characterized as recent and old alluvium soils (typic endoaquepts) with sandy to sandy-loam texture (sand 57.18%, silt 18.16%, clay 27.29%) and slightly to moderately acidic soil pH (5.4) with bulk density of  $1.24 \text{ Mg m}^{-3}$ , porosity 37.05%, water holding capacity 46.26%, and soil organic carbon  $10.68 \text{ g kg}^{-1}$ , available N, available phosphorous and available potassium contents of  $157.15 \text{ kg ha}^{-1}$ ,  $31.66 \text{ kg ha}^{-1}$ , and  $225.79 \text{ kg ha}^{-1}$ , respectively. The total rainfall recorded during the experimental period (April to August) was 250.14 mm in 2014 and 1508.71 mm in 2015.

The main field was ploughed, puddled thoroughly to 15-cm depth and levelled. Six treatments of PGRs were applied in the plant in a randomized block design with four replications. The treatments were i) control (CNTL), ii) abscisic acid (ABA,  $20 \text{ mg L}^{-1}$ ), iii) kinetin (KIN,  $10 \text{ mg L}^{-1}$ ), iv) kinetin (KIN,  $20 \text{ mg L}^{-1}$ ), v) cytozime (CYT,  $20 \text{ mg L}^{-1}$ ), and vi) wet tea extract (TE, 1:20 w/w) and the treatments were applied at tillering and panicle initiation stages of crop. The plots (size;  $4 \text{ m} \times 4 \text{ m}$ ), were prepared, with a gap of 0.5 m between two plots. High yielding rice variety Lachit was selected for this study. 30 days old seedlings of rice variety Lachit were transplanted (two seedlings per hill) to the experimental plots in April 2014 and 2015 at a spacing of  $20 \times 15 \text{ cm}$  (row  $\times$  plant). The crop was harvested in the month of August 2014 and 2015.

### 2.3. Fertiliser application and irrigation schedule

The fertiliser NPK was applied at the rate of  $40:20:20 \text{ kg of N-P}_2\text{O}_5\text{-K}_2\text{O ha}^{-1}$  in the form of urea (N), single super phosphate (P), and muriate of potash (K) as recommended by Agriculture Department of Government of Assam, India. The 1/3rd of N (as urea,  $13.33 \text{ kg ha}^{-1}$ ) was applied as a basal dose by broadcasting before the last ploughing in all the treatments. The remaining portion of N 1/3rd ( $13.33 \text{ kg ha}^{-1}$ ) was applied at 21–28 days after transplanting (DAT) of the crop and the other 1/3rd ( $13.33 \text{ kg ha}^{-1}$ ) of N was applied at 56–70 DAT of the crop. The whole quantity of  $\text{P}_2\text{O}_5$  ( $20 \text{ kg ha}^{-1}$ ) and  $\text{K}_2\text{O}$  ( $20 \text{ kg ha}^{-1}$ ) were applied before transplanting and mixed thoroughly with soil in all the treatments. Irrigation was done before transplanting of the crop. Consistent with local farmers' practice irrigation (four numbers) were done up to 7 days after transplanting and then the crop was allowed to grow depending only on rain water.

### 2.4. Foliar application of PGRs

The solutions of ABA ( $20 \text{ mg L}^{-1}$ ) and KIN ( $10 \text{ mg L}^{-1}$  and  $20 \text{ mg L}^{-1}$ ) were prepared by dissolving in a small volume of 1 normal (1 N) methanol and ethanol, respectively due to their insolubility in water. Then the solutions were brought to the required volumes of 2000 ml by adding distilled water for foliar spray on rice leaves. Tea extract was prepared by extracting the used tea leaves with distilled water (1:20 w/w) and the extract was filtered through 300 mesh screen (Yin et al., 2009). The volume of CYT and TE were also made up to 2000 ml with distilled water in order to get the desired concentration of  $20 \text{ mg L}^{-1}$ . The prepared solution of growth regulators were then sprayed on the rice leaves at the tillering stage and panicle initiation stage with a back-pack sprayer system (Ghodrat et al., 2012). Treated plants were sprayed completely and homogeneously with solutions (young and old leaves). Control plants were treated with distilled water and spraying was done in a clear and calm day during the morning hours. Sufficient care was taken to prevent the hormonal solution from dripping into the surface of the soil.

## 2.5. Fourier transform infrared spectrophotometer (FTIR) study

For FTIR analysis, tea leaf sample was air dried and 10 mg of the dried sample was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The discs so prepared containing the sample, was loaded into a FTIR spectrophotometer (Spectrum 1000, Perkin Elmer, USA) with a scan range from 4000 to 500  $\text{cm}^{-1}$ , with a wavelength accuracy of  $\pm 1$  nm and wavelength reproducibility of  $\pm 0.5$  nm. Three samples were analyzed in FTIR and mean values are presented.

## 2.6. Gas sampling and measurements

The fluxes of  $\text{N}_2\text{O}$  were measured at 7-day intervals on the basis of the static chamber technique and gas chromatography methods (Wang et al., 2011; Baruah et al., 2012). The chambers of 50-cm length, 30-cm width, and 90-cm height made of 6 mm thick acrylic transparent sheets were used for gas sampling. In each sampling plot, U-shaped aluminum channels (50 cm  $\times$  30 cm) were inserted into the soil to a depth of 15 cm to accommodate the chambers. The chambers were placed on the channels at the time of sampling. During gas sampling, the aluminum channel was filled with water, which acted as an air seal when the chamber was placed on the channel. Air inside the chamber was thoroughly mixed/ homogenized with a battery operated fan before sampling. Air temperature inside the chamber was measured by using mercury thermometers while taking gas samples. Gas samples were collected from the chambers by an airtight syringe (50 ml volume) at an interval of 15 min (0, 15, 30, and 45 min) between 9:00 to 11:00 a.m. in the morning on every sampling day.  $\text{N}_2\text{O}$  fluxes were calculated from the linear increase in  $\text{N}_2\text{O}$  inside the chamber during the gas sampling period (Parashar et al., 1996). Gas samples were brought to the laboratory immediately after sampling and analyzed for  $\text{N}_2\text{O}$  concentrations using a gas chromatograph (Varian 3800, USA) fitted with an electron capture detector (ECD) and a stainless steel Chromopack capillary column (50-cm long, 0.53-mm outside diameter, 1- $\mu\text{m}$  inside diameter). The operating temperature of the column, injector, and detector of ECD were 80, 200, and 300  $^\circ\text{C}$ , respectively. The carrier gas was pure  $\text{N}_2$  (99.999%) with a flow rate of 15  $\text{ml min}^{-1}$ . GC response was calibrated using certified  $\text{N}_2\text{O}$  standard obtained from the National Physical Laboratory, New Delhi, India.

Seasonal cumulative  $\text{N}_2\text{O}$  emissions for the entire crop growth period were computed by the method given by Ma et al. (2009) by using the following equation:

$$\text{Cumulative } \text{N}_2\text{O emission} = \sum_{i=1}^n (R_i \times D_i)$$

where  $R_i$  is the mean gas emission,  $D_i$  is the number of days between two sampling intervals i.e., 7-day interval,  $n$  is the total number of measurements made during the experiment.

## 2.7. Global warming potential (GWP) and carbon equivalent emission (CEE)

GWP of each treatment was calculated from the cumulative flux using the following equation (Watson et al., 1996):

$$\text{GWP} (\text{kg CO}_2 \text{ eq. ha}^{-1}) = \text{N}_2\text{O} \times 298$$

Based on a 100-year time frame, the GWP coefficient for  $\text{N}_2\text{O}$  is taken as 298 (IPCC, 2007). The CEE of the treatments was calculated following the method of Bhatia et al. (2010) and Baruah et al. (2016):

$$\text{CEE} (\text{kg C ha}^{-1}) = \text{GWP} \times \frac{12}{44}$$

## 2.8. Plant morphological and physiological parameters

Tiller number and leaf area index (LAI) were recorded at tillering, panicle initiation, flowering, and maturation stages of crop growth. Five randomly selected plants from each replication were taken for tiller number counting ( $5 \times 4 = 20$ ). Total leaf area of plants within 1  $\text{m}^2$  area of the planted plots was estimated by a portable laser leaf area meter (CID model CI-203, Camas, WA, USA) from four replications. LAI was expressed as the total leaf area per unit ground area ( $\text{m}^2$ ). Leaf photosynthesis and transpiration were measured with an infrared gas analyzer (LI-6400, portable photosynthesis system, LI-COR, Lincoln, NE, USA). The middle portion of a fully expanded green leaf from the top of the canopy was used for measurement of photosynthesis. Three plants were randomly selected from each replication for this measurement and presented as a mean of 12 readings ( $4 \times 3 = 12$ ) of each treatment. After panicle initiation stage (56 DAT onwards), measurement of photosynthesis and transpiration rate was done by taking the fully expanded flag leaf.

## 2.9. Plant anatomical parameters

The stomatal frequency of flag leaf and xylem vessels size of the node attached to the flag leaf under different treatments were analyzed with the help of scanning electron microscope (SEM) at panicle initiation stage. Leaf sections were cut from the middle portion of the fully expanded flag leaf with a sharp blade and immediately fixed in 3% glutaraldehyde for 24 h at 4  $^\circ\text{C}$ . Primary fixation was followed by washing in 0.1 M sodium cacodylate buffer. Post-fixation was done by immersing the samples overnight in 1% osmium tetroxide for 2–4 h. Thereafter, the samples were washed again in 0.1 M sodium cacodylate buffer. Washing was followed by dehydration with a graded acetone series (30, 50, 70, 80, 90, 95, and 100 %-two changes of 15 min each) at 4  $^\circ\text{C}$ . Dehydrated samples were immersed in tetra methyl silane (TMS) for 5–10 min (two changes) at 4  $^\circ\text{C}$  and then brought to room temperature (26  $^\circ\text{C}$ ) for drying. The samples were then mounted on metal stubs with a carbon adhesive tape, and platinum sputter coating was done by Auto Fine Coater (JFC-1600; JEOL, Tokyo, Japan) before examining the samples under a SEM (JEOL Asia PTE Ltd., Singapore/JEOL JSM, and Model 6390 LV). Three random fields from each sample were taken for the measurement of stomatal frequency (from the adaxial surface), and average stomatal frequency was expressed as the number of stomata  $\text{mm}^{-2}$  of flag leaf area. For the measurement of xylem size, sections of the node attached to the flag leaf were prepared following the procedure described above. Four SEM micrographs were taken to measure the internal diameter of the xylem vessels of a particular treatment. The internal diameter of all xylem vessels present in the micrograph covering the micrographic field was calculated in  $\mu\text{m}$  according to the scale visible on the micrographs and the average diameter of all xylem vessels was considered as xylem vessels size. Observations and micrographs with the SEM were made at 20 kV and 1000 $\times$  magnifications.

## 2.10. Soil samples analysis

Prior to rice cultivation soil samples were collected randomly from different locations of the experimental field from a depth of



0–15 cm and samples were analyzed for the basic physicochemical properties by following the methods of Page et al. (1982).

### 2.11. Estimation of yield

Grain yield was recorded by harvesting the rice from 1 square meter ( $\text{m}^2$ ) area from each replication ( $n = 4$ ). The grains were separated from the straw, dried, and weighed.

### 2.12. Statistical analysis

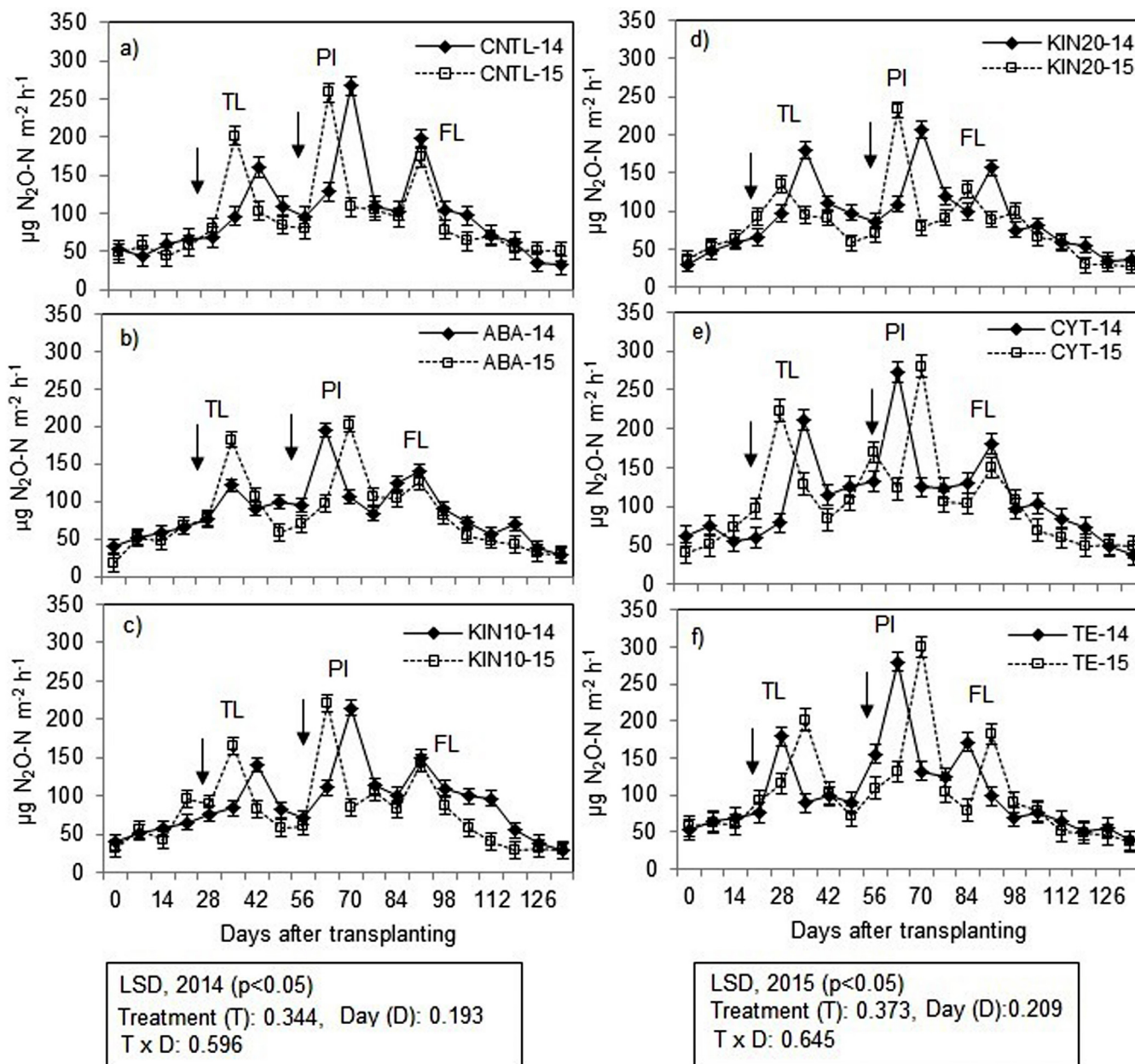
Statistical analysis was done to identify the significant differences among the treatments for different parameters after two years of experiment. Statistical analysis of data was performed by

Analysis of Variance (ANOVA), Pearson correlation analysis, Least Significant Difference (LSD), Duncan multiple range test (DMRT) with the help of SPSS analytical tool (IBM SPSS 20, SPSS Inc., Chicago, USA). Pearson-correlation analysis was conducted to determine the significance of linear relationships between obtained variables. Significant differences among the treatments for two years of experimentation were done by using two-way ANOVA and DMRT.

## 3. Results and discussion

### 3.1. Variation of $\text{N}_2\text{O}$ emissions from rice field

The  $\text{N}_2\text{O}$  emission rates from pre-monsoon rice ecosystems



**Fig. 1.** Nitrous oxide flux ( $\mu\text{g m}^{-2}\text{h}^{-1}$ ) from rice field in 2014 and 2015. Arrows indicate the time of fertiliser application; vertical bars represent standard error of mean. (CNTL, control; ABA, abscisic acid  $20 \text{ mg L}^{-1}$ ; KIN10, kinetin  $10 \text{ mg L}^{-1}$ ; KIN20, kinetin  $20 \text{ mg L}^{-1}$ ; CYT, cytozyme  $20 \text{ mg L}^{-1}$ ; TE, tea extract; TL, tillering; PI, panicle initiation; FL, flowering; LSD, least significant difference).

**Table 1**

Cumulative N<sub>2</sub>O emission, global warming potential, carbon equivalent emission and grain yield under different treatments compared by two-way ANOVA. Different small letters within the same column indicate differences among the treatments at <0.05 level by Duncan's multiple rang test. The  $\pm$  value indicates the standard error of mean.

Treatments	Cumulative N <sub>2</sub> O emission (mg N <sub>2</sub> O m <sup>-2</sup> )	Emission reduction over control (%)	Global warming potential (kg CO <sub>2</sub> eq. ha <sup>-1</sup> )	Carbon equivalent emission (kg C ha <sup>-1</sup> )	Yield (q ha <sup>-1</sup> )
CNTL	299.70 $\pm$ 2.17c		893.11 $\pm$ 6.48c	243.57 $\pm$ 3.06c	24.02 $\pm$ 0.73c
ABA	265.61 $\pm$ 4.72e	11.37	791.52 $\pm$ 14.08e	215.87 $\pm$ 3.84e	24.93 $\pm$ 0.80bc
KIN 10	273.68 $\pm$ 9.11d	8.68	815.57 $\pm$ 17.17d	222.43 $\pm$ 7.41d	26.18 $\pm$ 1.21a
KIN 20	275.44 $\pm$ 6.72d	8.09	820.81 $\pm$ 20.04d	223.86 $\pm$ 5.46d	25.52 $\pm$ 0.54 ab
CYT	346.55 $\pm$ 4.16a	Ns	1032.72 $\pm$ 12.42a	281.65 $\pm$ 3.39a	26.27 $\pm$ 0.80a
Tea extract	324.28 $\pm$ 1.39b	Ns	966.35 $\pm$ 7.20b	263.55 $\pm$ 1.96b	24.61 $\pm$ 0.93bc
P value, Treatment (T)	0.000		0.000	0.000	0.004
Year (Y)	0.000		0.000	0.000	0.000
T $\times$ Y	0.000		0.001	0.000	0.017

CNTL, control; ABA, abscisic acid 20 mg L<sup>-1</sup>; KIN10, kinetin 10 mg L<sup>-1</sup>; KIN20, kinetin 20 mg L<sup>-1</sup>; CYT, cytozyme 20 mg L<sup>-1</sup>; TE, tea extract Ns, not significant.

treated with PGRs namely ABA, KIN (10 and 20 mg L<sup>-1</sup>), CYT and TE as well as from the control are presented in Fig. 1. The N<sub>2</sub>O fluxes ranged from 28.56 to 299.35  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup>h<sup>-1</sup> in 2014 and from 16.89 to 298.90  $\mu$ g N<sub>2</sub>O-N m<sup>-2</sup>h<sup>-1</sup> in 2015 in response to different treatments (Fig. 1). The N<sub>2</sub>O emission increased gradually, and the first prominent peaks of N<sub>2</sub>O were observed at 35–42 DAT (tillering stage) during 2014 and 2015. The occurrence of emission peaks at the tillering stage may have been because of increased growth and development of the rice plants, manifested as an increase in the canopy size or expansion of leaf-blade surface area (Aulakh et al., 2000). Irrespective of the treatments, the highest N<sub>2</sub>O emission peaks were observed at 63–70 DAT (panicle initiation stage), and at this stage, the CYT and TE-treated plants exhibited higher N<sub>2</sub>O emission rates than did the plants receiving other treatments and the control during both the years (Fig. 1). The observed high emission rates at this stage may be attributed to an additional increase in leaf surface area with the emergence of flag leaves. The application of the second and third doses of nitrogenous fertiliser at tillering and panicle initiation may also have contributed to the emission peaks recorded in response to the treatments. Similar patterns of N<sub>2</sub>O emission after fertiliser application were reported by Zhang et al. (2015) and Bordoloi et al. (2016) in rice. Minor emission peaks were observed in all the treatments at 84–91 DAT (flowering stage) during both the years. The N<sub>2</sub>O fluxes gradually reduced from 112 DAT to harvest; the fluxes were minimum at harvest (Fig. 1) irrespective of the treatments. The reduction in N<sub>2</sub>O fluxes recorded at the later stages of crop growth is primarily caused by reduction in tiller number, leaf senescence, and the low availability of substrates for N<sub>2</sub>O production. Our results are consistent with those of Das and Adhya (2014) and Bordoloi et al. (2016).

Cumulative N<sub>2</sub>O emission rates were significantly different ( $p < 0.05$ ) among the treatments during the study period (Table 1). The descending order of N<sub>2</sub>O emission rates was as follows: CYT (346.55 mg N<sub>2</sub>O m<sup>-2</sup>) > TE (324.28 mg N<sub>2</sub>O m<sup>-2</sup>) > control (299.70 mg N<sub>2</sub>O m<sup>-2</sup>) > KIN20 (275.44 mg N<sub>2</sub>O m<sup>-2</sup>) > KIN10 (273.68 mg N<sub>2</sub>O m<sup>-2</sup>) > ABA (265.61 mg N<sub>2</sub>O m<sup>-2</sup>). Our study indicates that N<sub>2</sub>O emission rates from CYT and TE-treated plants were significantly ( $p < 0.05$ ) higher over the control plants; however, N<sub>2</sub>O emission rates of the ABA, KIN10 and KIN20-treated plants were 11.37%, 8.68%, and 8.09%, respectively, lower than that by the control plants. (Table 1,  $p < 0.05$ ). The variation in N<sub>2</sub>O emission rates with PGR application might have been caused by changes in the hormonal control of growth, physiological processes, and the anatomical configuration of the rice plants. The associations of factors such as LAI, tiller number, leaf transpiration, and stomatal frequency with N<sub>2</sub>O emission rates are well documented (Chen et al., 2008; Baruah et al., 2010, 2012), and the results of the present study are consistent with the previous reports.

### 3.2. Global warming potential (GWP) and carbon equivalent emission (CEE)

The GWP was significantly ( $p < 0.05$ ) affected by the treatments, and it varied from 791.52 (ABA) to 1032.72 kg CO<sub>2</sub> eq. ha<sup>-1</sup> (CYT) (Table 1). When the N<sub>2</sub>O emission rates were expressed as aggregate CO<sub>2</sub>-equivalents, the CYT and TE-treated plants were the highest contributors to the GWP, and it was 15.63% and 8.2%, respectively, higher than that of the control plants. Similarly, CEE values ranged from 215.87 (ABA) to 281.65 kg C ha<sup>-1</sup> (CYT) depending on the treatments. The CEE values of the plants treated with ABA (215.87 kg C ha<sup>-1</sup>), KIN10 (222.43 kg C ha<sup>-1</sup>), and KIN20 (223.86 kg C ha<sup>-1</sup>) were lower than those of the control plants (243.57 kg C ha<sup>-1</sup>) (Table 1). N<sub>2</sub>O emission rates in response to different PGR treatments were converted to CO<sub>2</sub> equivalents by using GWP, which gives an understanding of the agricultural impact on radiative forcing. Higher cumulative N<sub>2</sub>O emission recorded from plants treated with CYT and TE resulting in higher GWP and CEE compared to other PGRs and control are in accordance with the results of Baruah et al. (2016). Plants treated with KIN and ABA exhibited significantly lower GWP and CEE than did the control plants and the plants receiving other treatments (Table 1). Application of KIN and ABA could be an effective measure for reducing agricultural N<sub>2</sub>O emission and thus mitigate the adverse impact of climate change (Datta and Adhya, 2014).

### 3.3. FTIR study of tea sample

The FTIR spectra of tea leaves sample are shown in Fig. 2. The FTIR bands for tea leaves sample showed strong bands at 3394.6 cm<sup>-1</sup>, 2921.9 cm<sup>-1</sup> and 1642 cm<sup>-1</sup>, which indicate O–H stretching of hydroxyl groups, C–H vibration (stretching) of aliphatic groups, and C=O stretching of amide I and carboxylic acid, respectively. Another broad band was noted between 1430 and 1455 cm<sup>-1</sup>, arising from the O–H in-plane band of carboxylic acids and the C–O stretch vibration of carbonates (Carballo et al., 2008). Another broad band that was noted at 1370 cm<sup>-1</sup> was attributed to C–H deformation in the methyl groups. The band at approximately 1239 cm<sup>-1</sup> was attributed to C–H stretch and O–H deformation of carboxyl groups. The band at 612.7 cm<sup>-1</sup> corresponded to primary and secondary amines and amides (from theobromine and caffeine). The FTIR spectroscopic results showed distinct absorption bands of functional groups, such as O–H, C=O, and C–H, which indicate that TE contains phytochemicals viz. polyphenols and caffeine (Khalil et al., 2014).

### 3.4. Effect of PGRs on tiller number and leaf area index

Exogenous application of PGRs (ABA, KIN, and CYT) and TE

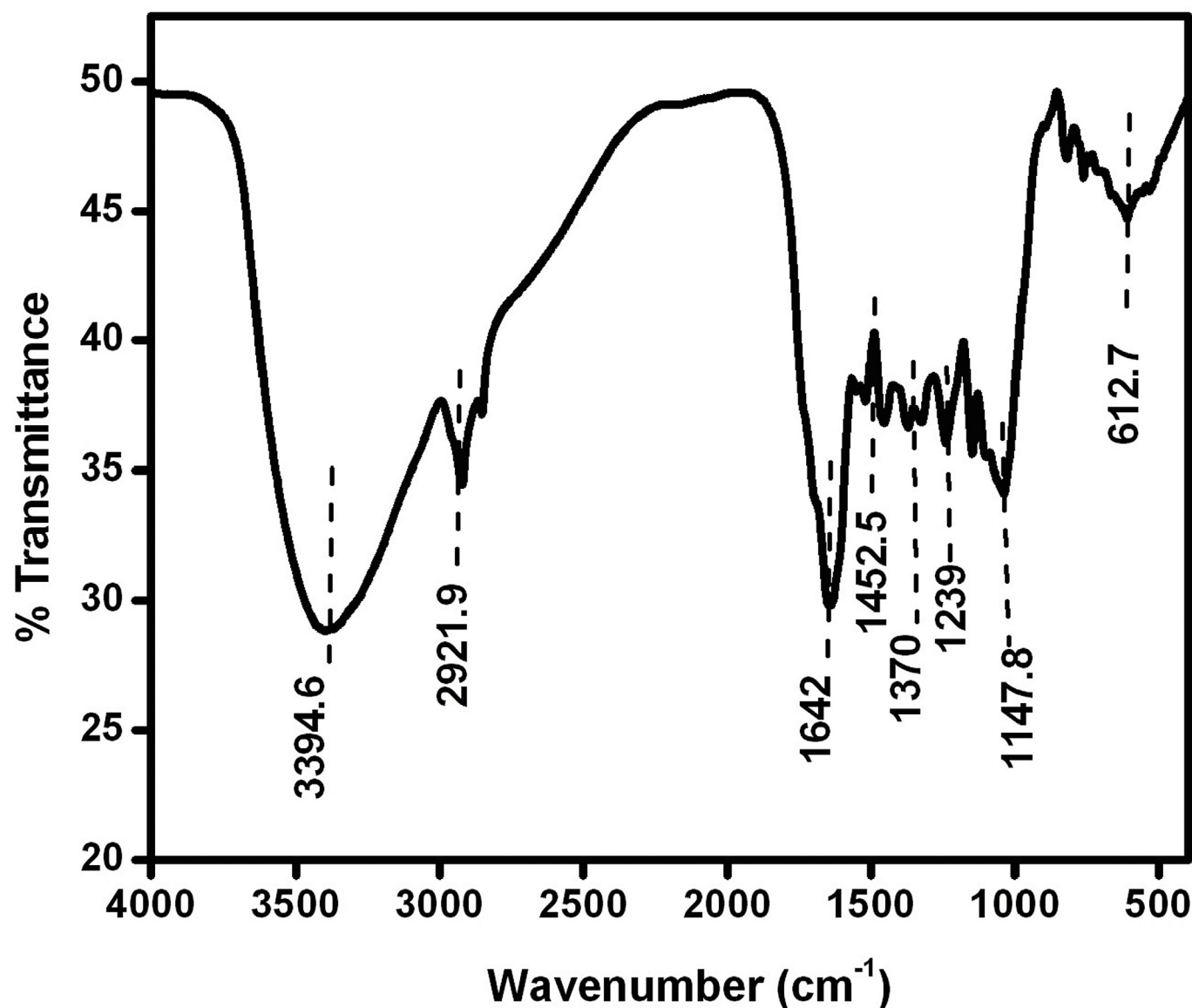
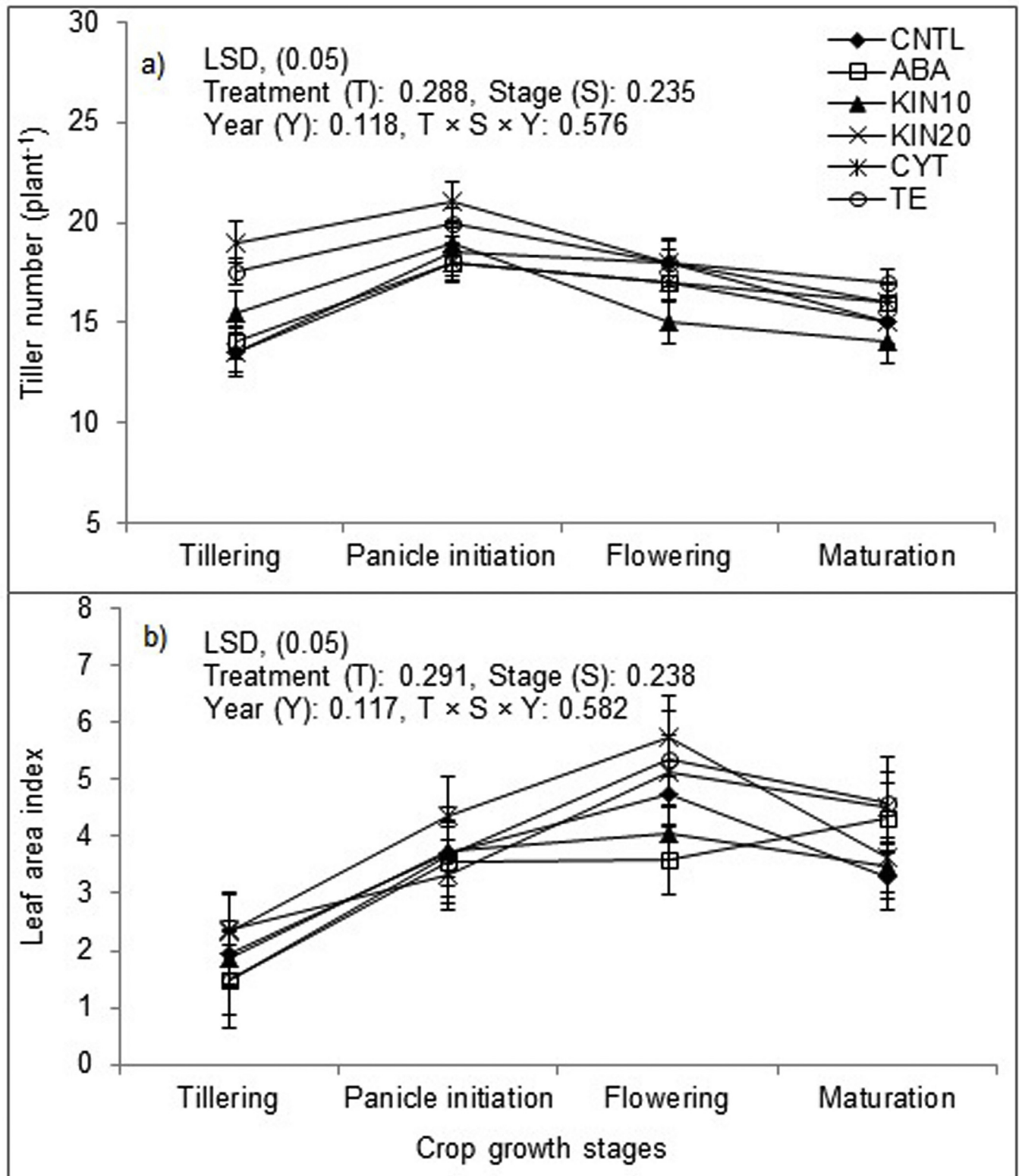


Fig. 2. FTIR analysis of tea sample.

regulated the growth and development of rice plants. Plant hormones are reported to play a crucial role in regulating tiller occurrence in rice plants (Liu et al., 2012). In the present investigation, the average number of tillers per plant ranged from 14 to 21 over the crop-growing season (Fig. 3a). Irrespective of the treatments, the number of tillers (19–21) recorded were higher at panicle initiation stage. Significant differences in tiller numbers were observed among the treatments during crop growth (Fig. 3a), and TE and CYT- treated plants recorded a higher tiller number than the control plants and the plants receiving other treatments. The tiller numbers remained fairly constant during the flowering stage of crop growth and then decreased slightly towards crop maturity because of degeneration of certain non-bearing tillers (Mohapatra and Kariali, 2008). The LAI values of rice plants after application of PGRs and TE ranged from 1.46 to 5.75 (Fig. 3b). Irrespective of the treatments, LAI was at its maximum at flowering stage of crop growth. Subsequently, the LAI values reduced with gradual decaying and senescence of leaves at crop maturity. At the flowering stage, the highest LAI values of 5.75 and 5.36 were observed in CYT

and TE- treated plants, followed by the LAI values of other treatments, namely KIN20 (5.14), control (4.75), KIN 10 (4.04), and ABA (3.58). An average, CYT and TE application was found to bring about an increase in LAI, whereas ABA application resulted in reduction of LAI (Fig. 3b).

Cytosyme is a plant growth stimulator, contains growth-promoting substances like gibberellic acid, auxins, seaweed extract, and trace elements (Gemici et al., 2000; Abubakar et al., 2013). The presence of such substances might have contributed to an increase in plant height, tiller number, and LAI. Auxins and gibberellins play a vital role in regulating the developmental processes of plant through cell elongation and by increasing internodal length (Rastogi et al., 2013). Similar mechanisms might have increased the tiller number and leaf area in CYT- treated rice plants. The phytochemicals present in the TE function as antioxidant, and they have been reported to exhibit hormonal-like properties. This activity might have increased tiller number and leaf area in the TE-treated plants (Andresen and Cedergreen, 2010; Tariq and Reyaz, 2012). KIN and ABA did not significantly increase the LAI in the



**Fig. 3.** Results of a) Tiller number and b) Leaf area index (LAI) at different stages of crop growth. (CNTL, control; ABA, abscisic acid 20 mg L<sup>-1</sup>; KIN10, kinetin 10 mg L<sup>-1</sup>; KIN20, kinetin 20 mg L<sup>-1</sup>; CYT, cytozyme 20 mg L<sup>-1</sup>; TE, tea extract; LSD, least significant difference).



**Table 2**  
Correlation matrix between plant parameters and N<sub>2</sub>O emission in rice field.

Parameters	Correlation with N <sub>2</sub> O emission (mg N <sub>2</sub> O m <sup>-2</sup> )
Tiller number (plant m <sup>-1</sup> )	0.714*
Leaf area index	0.625*
Photosynthesis (μ mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	0.158 ns
Transpiration rate (m mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.530*
Stomata frequency (stomata m m <sup>-2</sup> of leaf area)	0.928**
Xylem vessel size (μm)	0.829**

ns, not-significant.

Levels of significance \*\*, P < 0.01 level (2-tailed); \*, P < 0.05 level (2-tailed).

present study. Development and expansion of leaf area because of cytokinins depends on its concentration and environmental conditions (Leite et al., 2003), and these factors possibly but not conclusively cause ineffective leaf expansion in rice plants. Higher LAI values and leaf stomatal frequency (Table 3, number of stomata per unit leaf area) in CYT and TE-treated plants might have resulted in higher N<sub>2</sub>O emission. The stomata play a pivotal role in gas and water vapour exchange between plants and the atmosphere (Xu and Zhou, 2008), and is an agreement with the findings reported herein. The correlation (Table 2) observed among N<sub>2</sub>O emission rates, tiller number, and LAI support the effect of PGRs on plant growth parameters, which cause variations of N<sub>2</sub>O emission rates. Our results are consistent with those reported by Cheng et al. (2006); Bordoloi et al. (2016) and Borah and Baruah (2016b), wherein the plant factors were reported to affect the N<sub>2</sub>O emission from rice paddies.

### 3.5. Effect of PGRs on leaf photosynthesis and transpiration rate

The rate of photosynthesis recorded at different critical growth stages of rice plants are shown in Fig. 4a, and the differences in photosynthetic rate was significant (p < 0.05) among the treatments. During the experimental period, the leaf photosynthetic rate ranged from 8.02 to 27.59 μ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. Irrespective of the treatments, the photosynthetic rate was higher at the panicle initiation stage; subsequently, the rate decreased towards crop maturity (Fig. 4a). An average, the plants treated with CYT, KIN10, and KIN20 recorded a higher photosynthetic rate than the control plants. The photosynthetic rates of plants treated with CYT, KIN10, and KIN20 were 19.06%, 18.83% and 17.67%, respectively, higher than the control plants. Several studies have reported that cytokinins stimulate the synthesis of leaf chlorophyll and enzyme activities and directly influence chloroplast composition and ultrastructure, thus leading to an increasing in the leaf photosynthetic rate (Aldequy et al., 2014; Vercruyssen et al., 2015). A similar mechanism might have caused increased leaf photosynthesis by KIN (10 and 20 mg L<sup>-1</sup>) in the present study. Our results of

the enhancement of leaf photosynthetic rate in rice after KIN application are consistent with those reported by Prokopova et al. (2010) and Borah and Baruah (2016b), wherein a KIN-induced increase in photosynthetic rate were reported in lettuce and rice plants, respectively. The enhancement of photosynthetic rate in CYT-treated plants may be due to a higher CO<sub>2</sub> assimilation rate (Reddy and Kumar, 1996) or because of an increase in the chlorophyll content as reported by Gemici et al. (2000). Exogenous ABA application reduced leaf photosynthetic rate in rice ecosystems (Fig. 4a). The inhibitory effect of ABA on photosynthesis might be because of stomatal limitation (Li and Xu, 2014) or the regulation of the activities of the photosynthetic enzymes by ABA (Hu et al., 2013). Our results of reduced stomatal frequency due to ABA application (Table 3) are consistent with those reported by Hu et al. (2013) and Li and Xu (2014). The leaf photosynthetic rate was weakly correlated with the N<sub>2</sub>O emission rate measured during the rice-growing period (Table 2), and results are in good agreement with the findings of Jiang et al. (2016).

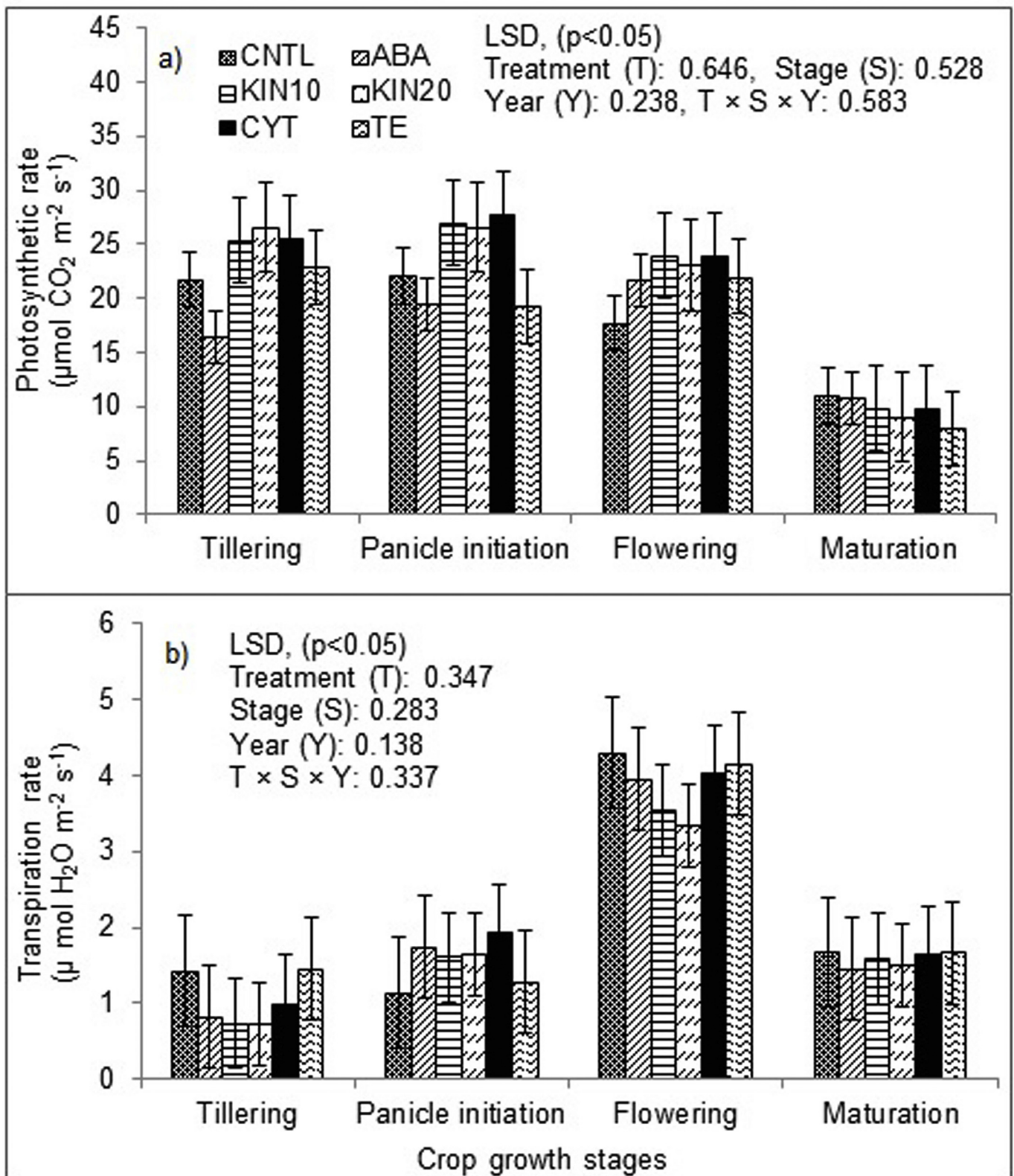
A combined analysis of leaf transpiration rates for both the years is presented in Fig. 4b. The rate of transpiration gradually increased from the initial stage up to the flowering stage and declined from the flowering stage onwards (Fig. 4b). The rate of transpiration in the PGR-treated plants varied from 0.729 to 4.3 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, and on average the control plants and TE- and CYT-treated plants exhibited a higher transpiration rate than did the KIN (10 and 20 mg L<sup>-1</sup>) and ABA-treated plants (Fig. 4b). By contrast, the leaf transpiration rates of ABA, KIN10 and KIN20 -treated plants were 9.67%, 15.12%, and 17.84%, respectively, lower than the control plants. Hormonal regulation of stomatal conductance, which directly or indirectly affects the leaf transpiration rate, was also reported by Zhang et al. (2008). Our results of variation in leaf transpiration (p < 0.05) in rice plants due to PGRs are well corroborated with the findings of Zhang et al. (2008) and Li and Xu (2014). The increased transpiration rate may have facilitated more transport of N<sub>2</sub>O to the atmosphere by CYT and TE-treated plants than by the plants receiving other treatments and the control plants. We also observed a positive relationship between the rate of transpiration and N<sub>2</sub>O emission rate (Table 2). A similar relationship between leaf transpiration rate and N<sub>2</sub>O emission was reported by Chang et al. (1998); Pihlatie et al. (2005) and Borah and Baruah (2016a) and the results of the present study support their findings. ABA and KIN were reported to play a crucial role in regulating leaf transpiration rate, which may directly or indirectly affect the transport of GHGs (Li and Xu, 2014). In the present study, KIN reduced the transpiration rate of rice plants, resulting in lower N<sub>2</sub>O emission rates than the control plants, and our results are consistent with the findings of Ozyigit and Can (2009) and Borah and Baruah (2016b). ABA is reported to inhibit the leaf transpiration (Hu et al., 2013; Li and Xu, 2014) and serve as an anti-transpirant (Pospisilova, 2003), which is parallel to our results of reduced leaf transpiration in ABA-treated plants.

**Table 3**  
Stomatal frequency of flag leaf and xylem size of node of rice under different treatments. Different small letters within the same column indicate differences among the treatments at <0.05 level by Duncan's multiple rang test. The ± value indicates the standard error of mean.

Treatments	Stomatal frequency (stomata mm <sup>-2</sup> of leaf area)	Xylem size (μm)
CNTL	723.40 ± 2.4c	24.25 ± 0.96b
ABA	621.27 ± 6.1f	21.66 ± 0.72c
KIN 10	680.85 ± 1.8e	24.00 ± 1.02b
KIN 20	697.87 ± 2.7d	24.33 ± 0.38b
CYT	808.51 ± 2.4b	28.33 ± 0.72a
TE	829.78 ± 1.2a	29.50 ± 1.06a
P value, Treatment (T)	0.025	0.005
LSD	0.825	0.822

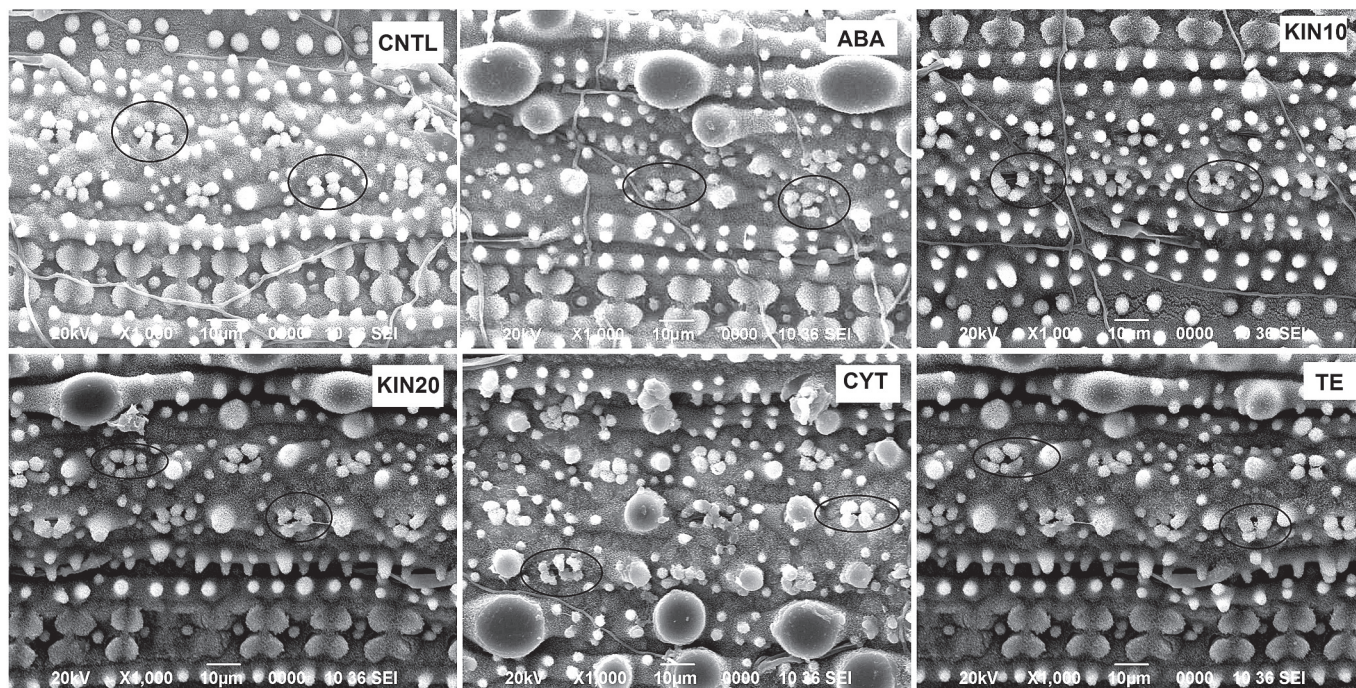
CNTL, control; ABA, abscisic acid 20 mg L<sup>-1</sup>; KIN10, kinetin 10 mg L<sup>-1</sup>; KIN20, kinetin 20 mg L<sup>-1</sup>; CYT, cytozyme 20 mg L<sup>-1</sup>; TE, tea extract.





**Fig. 4.** a) Plant photosynthetic rate and b) Transpiration rate at different stages of crop growth. Vertical bars represent standard error of mean. (CNTL, control; ABA, abscisic acid  $20 \text{ mg L}^{-1}$ ; KIN10, kinetin  $10 \text{ mg L}^{-1}$ ; KIN20, kinetin  $20 \text{ mg L}^{-1}$ ; CYT, cytozyme  $20 \text{ mg L}^{-1}$ ; TE, tea extract; LSD, least significant difference).



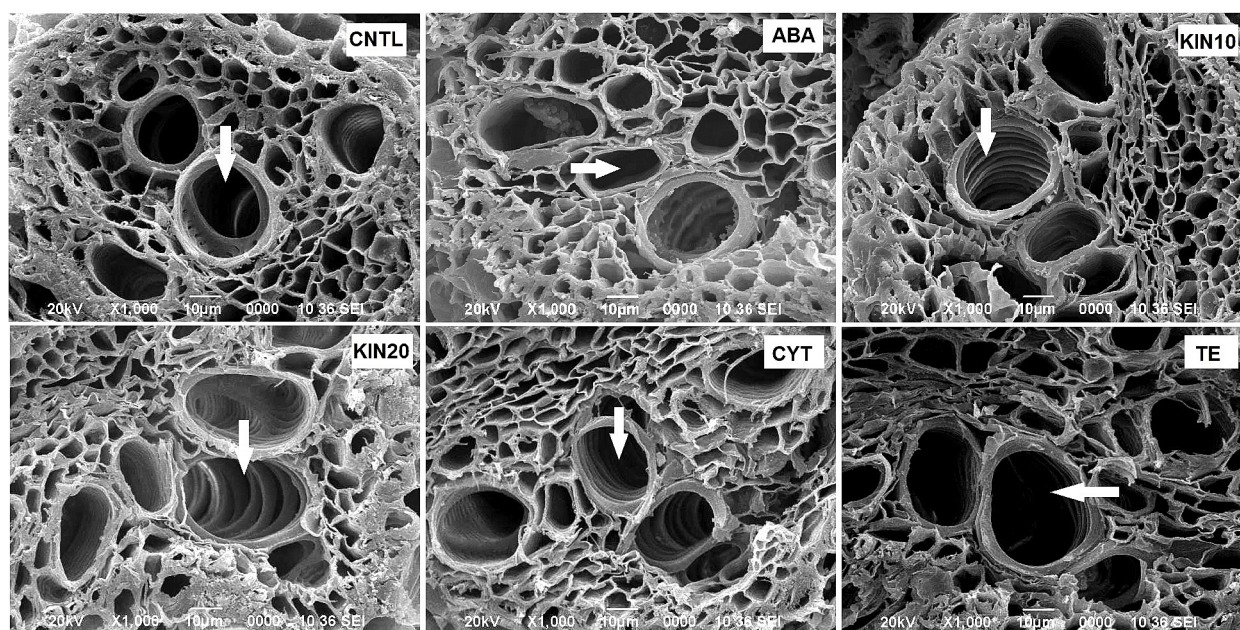


**Fig. 5.** Scanning electron micrographs of adaxial surface of flag leaf, circled areas indicate stomata of leaf. (CNTL, control; ABA, abscisic acid 20 mg L<sup>-1</sup>; KIN10, kinetin 10 mg L<sup>-1</sup>; KIN20, kinetin 20 mg L<sup>-1</sup>; CYT, cytozime 20 mg L<sup>-1</sup>; TE, tea extract).

### 3.6. Plant anatomical parameters

The scanning electron micrographs of the adaxial surface of flag leaf of plants from different treatments are presented in Fig. 5. Results of analysis of variance revealed a significant difference in stomatal frequency among the treatments (Table 3). The flag leaf stomatal frequency of TE and CYT-treated plants was higher (829.78 and 808.51 stomata mm<sup>-2</sup> of leaf area, respectively) than that of the control plants (723.40 stomata mm<sup>-2</sup> of leaf area). By

contrast, the plants treated with ABA (621.27 stomata mm<sup>-2</sup> of leaf area), KIN10 (680.85 stomata mm<sup>-2</sup> of leaf area), and KIN20 (697.89 stomata mm<sup>-2</sup> of leaf area) exhibited lower stomatal frequency than did the control plants. Reduced leaf area development in ABA and KIN-treated plants contributed to a reduction in leaf stomatal frequency in these plants (Table 3). N<sub>2</sub>O has been reported to be released through leaf stomata; therefore, leaf stomatal frequency is likely to control N<sub>2</sub>O emission to the atmosphere. ABA and KIN application reduced the N<sub>2</sub>O emission by regulating the stomatal



**Fig. 6.** Scanning electron micrographs of node attached to flag leaf, arrows point to the individual xylem vessels. (CNTL, control; ABA, abscisic acid 20 mg L<sup>-1</sup>; KIN10, kinetin 10 mg L<sup>-1</sup>; KIN20, kinetin 20 mg L<sup>-1</sup>; CYT, cytozime 20 mg L<sup>-1</sup>; TE, tea extract).



frequency of the leaves of the rice plants (Table 3). Conversely, increased LAI in CYT and TE-treated plants might have resulted in a higher stomatal frequency than in the control plants, resulting in higher N<sub>2</sub>O emission rates. High LAI values, stomatal frequency, and transpiration rate may result in high N<sub>2</sub>O emission rates; this finding is further supported by the positive relationship of the transpiration rate ( $r = 0.530$ ,  $p < 0.05$ ) and stomatal frequency ( $r = 0.928$ ,  $p < 0.01$ ) with N<sub>2</sub>O emission in the present study (Table 2) and results are well corroborated with Xu and Zhou (2008) and Borah and Baruah (2016a).

The scanning electron micrographs of the xylem vessels of the node are shown in Fig. 6. The size of xylem vessels is associated with the process of transpiration and also mediates the process of gas exchange to the atmosphere (Loepfe et al., 2007). Significant differences in the xylem vessel size were observed in the rice plants (Table 3) because of the application of PGRs. ABA was most effective in reducing the xylem vessel size (21.66  $\mu\text{m}$ ) of the node followed by KIN10 (24  $\mu\text{m}$ ) and KIN20 (24.33  $\mu\text{m}$ ), compared with the control (24.75  $\mu\text{m}$ ); however, the difference between the size of the xylem vessels of the KIN10 and KIN20-treated plants was not prominent. The internal diameter of the xylem vessels was higher in CYT and TE-treated plants (28.33 and 29.50  $\mu\text{m}$ , respectively) than in the other treatments and control plants. Moreover, they also exhibited higher seasonal N<sub>2</sub>O flux over others treatments and control plants, thus suggesting the role of xylem size in the process of N<sub>2</sub>O transport and emission. ABA and KIN treatments reduced xylem vessels size in the rice plants that exhibited low N<sub>2</sub>O emission rates. In our study, the regulation of xylem vessel size by PGRs was mediated through modification of the vascular cell differentiation and pattern of vascular tissue formation in plants (Sorce et al., 2013). The application of different growth regulators may alter the plant vascular characteristics such as xylem size, which in association with leaf transpiration, affect N<sub>2</sub>O emission rates. The mechanism of GHG (CH<sub>4</sub>) transport through xylem vessels was reported by Carmichael et al. (2014) in woody herbaceous plants and Borah and Baruah (2016b) in rice plants. Our findings of the relationship between xylem vessel size and N<sub>2</sub>O transport in rice are consistent with the findings reported by Carmichael et al. (2014) and Borah and Baruah (2016b). This concept is further strengthened by the observed correlation ( $r = 0.829$ ,  $p < 0.01$ ) of xylem vessel size and leaf transpiration with N<sub>2</sub>O emission in the present study (Table 2).

### 3.7. Response of grain yield to PGRs

The grain productivity of the rice plants treated with PGRs was higher than that of the control plants. The yield of TE and ABA-treated plants were at par with the control (Table 1). Statistical analysis revealed that ( $p < 0.05$ ) the effects of treatments on economic yield were significant, and CYT and KIN (10 and 20 mg L<sup>-1</sup>) treatments increased grain productivity (Table 1). The grain yield of CYT-treated plants was 9.37% higher than the control; however, the N<sub>2</sub>O emission rates of the CYT-treated plants were the highest. The grain productivity of the KIN10 and KIN20-treated plants was higher than that of the control plants by 8.99% and 6.24%, respectively, coupled with lower N<sub>2</sub>O emission. Cytokinin may play a role in mediating cell division in the endosperm during the rice grain-filling stage and might regulate the sink size (grain capacity) (Hansen and Grossmann, 2000). A similar mechanism (reported by Hansen and Grossmann, 2000) might have caused an increase in grain yield in the KIN-treated plants. Efficient photosynthate partitioning may also contribute to high grain productivity in the CYT-treated plants, which is evident from the results of higher photosynthetic rates in CYT-treated plants (Fig. 4a). Regulating sink strength either by controlling assimilate transport to the sink or by

mediating the division and enlargement of endosperm cells in the KIN and CYT-treated plants are important factors, which influence grain productivity in the rice ecosystem. A similar source and sink relationship due to PGR application in rice was previously reported (Javid et al., 2011; Tiwari et al., 2011; Borah and Baruah, 2016b) and our results are well corroborated with these findings.

## 4. Conclusions

This study demonstrated that the application of ABA and KIN (10 and 20 mg L<sup>-1</sup>) significantly reduced cumulative N<sub>2</sub>O emission and GWP through regulation of the morphological, physiological, and anatomical characteristics of the rice plants. The cumulative N<sub>2</sub>O emission of ABA and KIN-treated (10 and 20 mg L<sup>-1</sup>) plants were 8%–11% lower than that of the control plants and TE and CYT increased the emission by 8%–16% over the control plants. Treatment with KIN and CYT improved the grain yield by increasing the efficient photosynthate translocation towards the developing grains in rice plants. Among the different PGRs used in the study, KIN (10 mg L<sup>-1</sup>) reduced N<sub>2</sub>O emission sustainably and simultaneously improved grain productivity. Considering the availability and market cost, KIN treatment (10 mg L<sup>-1</sup>) can be recommended for further application in rice fields for reducing N<sub>2</sub>O emission. However, we recommend additional multilocation studies involving KIN (10 mg L<sup>-1</sup>) application in rice fields.

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